STATE OF ALASKA
DEPT. OF HEALTH & SOCIAL SERVICES

DIVISION OF PUBLIC HEALTH
SECTION OF EPIDEMIOLOGY

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INFECTIOUS DISEASES
AIDS/STD
TUBERCULOSIS
IMMUNIZATION
CHRONIC DISEASES
DIABETES
INJURY CONTROL

(907) 269-8000
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Clinical Laboratory Tests for Anthrax

The Section of Epidemiology must review and approve all clinical anthrax tests prior to submission to the Alaska State Public Health Laboratories (ASPHL). Call:

907-269-8000 during business hours or
1-800-478-0084 after hours

I. Exposure to substance that could be anthrax

Currently there is no clinical laboratory test that reliably determines whether a patient has been exposed to anthrax spores. If exposure to anthrax is suspected, anthrax testing should be performed on the suspicious letter, package or other material to which the patient was exposed. When a patient has confirmed or probable exposure to anthrax spores, preventive treatment is indicated, regardless of the results of nasal swab testing.

A. Anthrax spore staining and culture

Procedure: Swab both nares using a dacron culturette system. (The Liquid Stuart Single Swab PK 50® is one example of such a system.) The ASPHL will stain the specimen and look for microscopic evidence of spores. The specimen is also placed into culture, which is read at 24 and 48 hours.

Interpretation of results: *Decisions about treatment for anthrax exposure or disease should not be made based on this test.* A positive anthrax spore stain or culture from someone exposed to anthrax spores indicates infection, but not disease. A negative test does not rule out infection. The nasal swab test is not a diagnostic tool because it is not standardized and may give false positive and negative results.

Availability: The Alaska State Public Health Laboratory-Anchorage (ASPHL-Anchorage) is currently the only laboratory in Alaska that can perform this test.

II. Evaluation of anthrax disease

During the early phase of *inhalation anthrax*, laboratory testing is frequently not helpful. Days after initial exposure, nasal swab testing for anthrax spores is usually negative. Blood cultures often do not become positive until the later stages of disease when treatment is much less effective. Because the anthrax organisms travel to the mediastinal lymph nodes and not the lungs, sputum cultures are rarely positive, even in the later stages of disease. In contrast to

inhalation anthrax, *cutaneous anthrax* can be diagnosed by special staining and culture of vesicular fluid, exudate or skin biopsy.

A. Culture

Procedure: Culture is the definitive test for anthrax. *Bacillus anthracis* can be isolated from blood, pleural fluid, CSF, ascitic fluid, vesicular fluid or skin lesion exudate. Growth of *B. anthracis* can be seen after 12-48 hours of incubation.

- Three **blood cultures** should be taken 10-30 minutes apart from separate sites prior to initiating antibiotic therapy. Blood should be collected directly into blood culture bottles incubated according to standard automated blood culture procedures. [1] If a facility does not have access to blood culture bottles, the blood can be collected in yellow-top tubes and sent to the ASPHL for culture.
- **CSF**, **pleural fluid**, and **ascitic fluid** should be collected in a sterile container. **Vesicular fluid** should be collected using a dacron swab culturette system. The specimen(s) is then planted on blood agar.
- **Sputum cultures** are rarely positive because the organism is concentrated in the mediastinal lymph nodes rather than in the lung parenchyma.
- When culturing a **skin lesion**, use dacron swab culturette to collect either vesicular fluid or exudate from the lesion. If there is no exudate visible, lift the edge of the eschar with a pair of forceps and collect fluid near the edge of the ulcer. A biopsy of the lesion can be taken for Gram stain, culture, and histology.

Interpretation of results: When submitting a specimen, it is important to alert the clinical laboratory that the diagnosis of anthrax is being considered; otherwise *Bacillus* species may be considered a contaminant and discarded. The ability for clinical microbiology laboratories to definitively identify *B. anthracis* is limited. Therefore, when considering the diagnosis of anthrax, any gram-positive bacillus that is non-hemolytic should be submitted to the ASPHL-Anchorage for identification.

Availability: Most clinical laboratories have the capacity to culture *B. anthracis*; however, ASPHL-Anchorage is the only laboratory in the state with the capacity to differentiate *B. anthracis* from other *Bacillus* species.

B. Microscopic techniques

1. **Anthrax spore staining**:

   Procedure: see ***1*.A**.

   Interpretation of results: The likelihood of detecting spores in the nares declines rapidly 24 hours after exposure. However, if inhalation anthrax disease is clinically suspected, this test may have some value in the early stages of disease, when blood cultures and chest x-ray are still negative. A negative test does not rule out disease.

[1] The Alaska State Public Health Laboratories does not have automated blood culture capacity at the present time. The local clinical laboratory should incubate blood cultures bottles. If growth occurs and gram positive bacilli are seen on gram stain, ASPHL will provide isolate identification.

Availability: see ***1*.A**.

2. **Gram stain**:

   Procedure: A Gram stain can be done on vesicular fluid or exudate from a cutaneous lesion, pleural fluid in suspected inhalation anthrax, and CSF for suspected meningeal anthrax. In advanced disease, unspun blood may be positive on gram stain. A Gram stain of *B. anthracis* shows large gram-positive bacilli singly or in short chains, with squared-off ends.

   Interpretation of results: As with any bacterial infectious disease, the Gram stain is a rapid screening tool, but not as sensitive or specific as the culture. A negative Gram stain does not rule-out anthrax.

   Availability: Gram stain can be performed at most clinical laboratories.

3. **Direct fluorescent antibody (DFA) test**:

   Procedure: This rapid staining technique can be used to examine exudate and tissue from cutaneous lesions and cerebral spinal fluid.

   Interpretation of results: A positive test is highly suggestive of anthrax. DFA is not usually helpful for inhalation anthrax because respiratory and pleural fluid is usually negative early in disease.

   Availability: This test is available at the ASPHL.

4. **Other rapid diagnostic tests**: An ELISA assay for antigen detection and PCR for detection of nucleic acid can give a preliminary diagnosis within hours. These tests are only available in certain circumstances through reference laboratories such as the Centers for Disease Control and Prevention in Atlanta.